Collaborative Study of the Rapid Determination of Moisture and Fat in the Same Sample of Meat or Meat Product

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A method utilizing azeotropic distillation for determining moisture and fat in the same sample of ground beef, frankfurters, or pork sausage was collaboratively studied. The apparent moisture content of these products, determined by measuring the volume of condensate after 30 min distillation with cumene, *m*-xylene, or ethylbenzene, was 1% lower than that determined by method 24.003(a). The fat content was determined by weighing the residue from an aliquot of the extract taken after 30 min (ground beef and frankfurters) or 45 min (pork) distillation. The fat content of ground beef and frankfurters, determined by using all 3 solvents, agreed with results obtained by method 24.005(a); results for pork samples were about 1% low. Significant positive correlation (r = 0.99) was obtained for both moisture and fat data with results from the AOAC methods. Statistical evaluation of the collaborative results for the 3 meat products, using the 3 solvents, led to expected standard deviations, s_e, of ±0.94% moisture and ±1.03% fat. Azeotropic distillation with cumene, *m*-xylene, or ethylbenzene is useful as a rapid screening procedure for determining moisture and fat in meats prior to processing or in finished products where rapid analysis is more important than maximum accuracy and precision.

Many analytical techniques are available for the determination of moisture or fat in meat and meat products (1). For quality control and regulatory compliance, meat processors need a method that will determine moisture and fat in the same sample; the method should be rapid, relatively accurate, and simple to perform. Use of available rapid methods has been limited because the procedures have not been developed fully or evaluated adequately. The AOAC methods (2), which serve as standards for the meat industry, are accurate but time consuming.

In seeking a suitable method, Cohen and Kimmelman (3) evaluated azeotropic distillation. For ground beef, frankfurters, and fresh pork sausage, the most suitable entrainer-extractants of the 27 different solvents tested were *m*-xylene and cumene. Pettinati *et al.* (1) reviewed the application of azeotropic distillation for the determination of moisture and fat in the food industry and concluded that this technique showed promise as a combined method which could be rapid, accurate, and relatively simple to perform for a low initial investment. This method allowed the moisture content to be measured directly as condensate volume in a calibrated receiver. The potential of the method, therefore, was tested by means of a collaborative study among 10 laboratories. Results were compared with those from analyses by the AOAC moisture and fat methods, **24.003(a)** and **24.005(a)**, respectively.

Experimental

Apparatus

The laboratory equipment and reagents needed for azeotropic distillation were described in ref. 4. Containers with the fat residue can be cooled in a desiccator before weighing. However, if rapid cooling is desired, a cooling chamber (5) is needed.

Preparation of Samples

Samples of ground beef, frankfurters, and pork sausage were taken from the same lots described in the first report of this series (4).

Moisture Determination by Azeotropic Distillation

For each determination, weigh 10.00±0.01 g sample and use 100.0±0.1 ml solvent in boiling flask. Use (*1*) cumene, (*2*) *m*-xylene, and (*3*) ethylbenzene (Baker reagent grade, if possible) for 6 replicate analyses of each sample, by each solvent, respectively. After 30 min distillation read volume of condensate to nearest 0.02 ml from receiver

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Table 1. Collaborative results for moisture analysis by azeotropic distillation in cumene for 3 meat products

	Moisture,[b] %						Ranked results			
	Ground beef		Frankfurter		Pork sausage					
Coll.[a]	$\bar{x}$	s_r	$\bar{x}$	s_r	$\bar{x}$	s_r	B	F	P	Coll. score
1(AR)	62.85	0.625	57.07	1.073	40.85	0.418	2	2	5	9
2	61.13	2.085	56.00	1.304	40.33	0.983	8	7	8	23
3	62.47	0.372	56.67	0.468	41.30	0.329	5	5	3.5	13.5
4	62.57	1.433	57.25	0.907	41.43	0.871	3.5	1	2	6.5
5	61.60	0.420	56.60	0.303	41.30	0.385	7	6	3.5	16.5
7	63.25	0.748	55.62	1.880	40.43	0.771	1	8	7	16
8	62.57	0.234	57.03	0.709	40.53	0.723	3.5	3	6	12.5
9	62.18	0.200	56.99	0.150	41.50	0.188	6	4	1	11
10	57.75	3.489	51.80	1.503	38.25	2.019	9	9	9	27

Statistic	Beef	Franks	Pork	Average
Intermediate Results, Per Cent Moisture				
Grand mean, $\bar{\bar{x}}$	61.82	56.11	40.66	—
Range	−10.82 to 2.18	−6.11 to 2.39	−4.66 to 1.54	—
s_d	1.653	1.704	1.009	1.456
s_r	1.488	1.070	0.906	1.155
s_b	1.537	1.647	0.939	1.375
Final Results,[c] Per Cent Moisture				
Grand mean, $\bar{\bar{x}}$	62.33	56.65	40.96	—
Range	−3.33 to 1.67	−4.65 to 1.85	−1.96 to 1.24	—
s_d	0.680	0.572	0.480	0.578
s_r	0.985	1.003	0.643	0.877
s_b	0.548	0.399	0.402	0.450

[a] Collaborator 6 did not report data for this solvent.
[b] 162 analyses.
[c] Data from Collaborator 10 omitted.

Table 2. Collaborative results for moisture analysis by azeotropic distillation in m-xylene for 3 meat products

	Moisture[b], %						Ranked results			
	Ground beef		Frankfurter		Pork sausage					
Coll.[a]	$\bar{x}$	s_r	$\bar{x}$	s_r	$\bar{x}$	s_r	B	F	P	Coll. score
1(AR)	62.37	0.606	56.50	0.429	41.40	0.358	3	5.5	4	12.5
2	61.00	0.707	55.88	0.850	40.75	0.689	8	8	5	21
3	62.47	0.450	56.67	0.301	41.63	0.446	1.5	3	3	7.5
4	62.47	0.677	57.63	1.347	40.64	0.731	1.5	1	6	8.5
5	61.92	0.279	56.82	0.184	42.40	0.283	6	2	1	9
7	61.63	0.554	56.38	0.392	40.08	1.348	7	7	8	22
8	62.20	0.219	56.50	0.352	40.40	0.790	4	5.5	7	16.5
9	62.12	0.272	56.55	0.241	41.66	0.170	5	4	2	11
10	60.33	1.211	53.70[c]	3.154	39.77	1.071	9	9	9	27

Statistic	Beef	Franks	Pork	Average
Intermediate Results, Per Cent Moisture				
Grand mean, $\bar{\bar{x}}$	61.83	56.29	40.97	—
Range	−2.83 to 1.77	−6.29 to 2.31	−2.47 to 1.73	—
s_d	0.733	1.076	0.856	0.889
s_r	0.624	1.125	0.748	0.833
s_b	0.687	0.951	0.800	0.813
Final Results,[d] Per Cent Moisture				
Grand mean, $\bar{\bar{x}}$	62.02	56.62	41.12	—
Range	−2.02 to 1.58	−1.62 to 1.98	−2.62 to 1.58	—
s_d	0.502	0.493	0.778	0.591
s_r	0.504	0.630	0.697	0.611
s_b	0.458	0.420	0.724	0.534

[a] Collaborator 6 did not report data for this method.
[b] 161 analyses.
[c] Collaborator 10 reported 5 replicate values only.
[d] Data from Collaborator 10 omitted.

Table 3. Collaborative results for moisture analysis by azeotropic distillation in ethylbenzene for 3 meat products

	Moisture,[a] %						Ranked results			
	Ground beef		Frankfurter		Pork sausage					
Coll.	$\bar{x}$	s_r	$\bar{x}$	s_r	$\bar{x}$	s_r	B	F	P	Coll. score
1(AR)	62.18	0.397	58.98	1.007	40.83	0.427	4	1	5	10
2	61.33	1.633	56.75	1.129	40.50	1.225	7	3.5	7	17.5
3	62.23	0.388	56.57	0.557	41.63	0.344	3	7	1	11
4	61.73	1.033	57.23	1.394	41.52	1.194	6	2	3	11
5	62.15	0.345	56.70	0.253	41.58	0.343	5	5	2	12
6	61.05	0.536	56.75	0.524	40.78	0.549	10	3.5	6	19.5
7	62.68	0.705	56.28	0.801	39.75	0.987	1	8	10	19
8	62.40	0.790	56.20	0.253	40.27	0.484	2	9	9	20
9	61.08	0.667	56.60	0.203	41.31	0.305	8.5	6	4	18.5
10	61.08	1.357	55.50[b]	0.707	40.33	0.876	8.5	10	8	26.5

Statistic	Beef	Franks	Pork	Average
Final Results, Per Cent Moisture				
Grand mean, $\bar{\bar{x}}$	61.79	56.76	40.85	—
Range	−2.79 to 2.21	−2.26 to 3.24	−1.85 to 1.75	—
s_d	0.615	0.904	0.646	0.722
s_r	0.886	0.786	0.756	0.810
s_b	0.498	0.833	0.567	0.633

[a] 178 analyses.
[b] Collaborator 10 reported 4 replicate values only.

Table 4. Collaborative results for fat analysis by azeotropic distillation in cumene for 3 meat products

	Fat,[a] %						Ranked results			
	Ground beef		Frankfurter		Pork sausage					
Coll.	$\bar{x}$	s_r	$\bar{x}$	s_r	$\bar{x}$	s_r	B	F	P	Coll. score
1(AR)	17.55	0.482	27.05	0.771	41.60	0.748	7	6	9	22
2	19.30	0.613	28.07	0.308	41.72	0.232	2.5	2	8	12.5
3	19.72	0.436	27.88	0.256	44.45	0.356	1	3	2	6
4	16.66	0.548	25.98	0.599	41.52	1.029	9	10	10	29
5	18.11	0.132	27.01	0.117	42.51	0.546	4	7	4	15
6	17.47	0.311	27.67	0.397	42.50	0.428	8	4	5	17
7	15.92	0.502	27.37	0.339	41.96	0.269	10	5	6	21
8	17.95	0.224	26.89	0.461	43.07	0.497	5	8	3	16
9	17.73	0.074	26.66	0.360	41.80	0.209	6	9	7	22
10	19.30	0.923	29.75	1.810	47.78	6.438	2.5	1	1	4.5

Statistic	Beef	Franks	Pork	Average
Intermediate Results, Per Cent Fat				
Grand mean, $\bar{\bar{x}}$	17.97	27.43	42.89	—
Range	−2.76 to 2.33	−2.04 to 5.27	−2.88 to 14.81	—
s_d	1.201	1.020	1.935	1.386
s_r	0.487	0.708	2.100	1.099
s_b	1.185	0.978	1.734	1.300
Final Results,[b] Per Cent Fat				
Grand mean, $\bar{\bar{x}}$	17.82	27.18	42.35	—
Range	−2.61 to 2.38	−1.79 to 1.22	−2.34 to 2.45	—
s_d	1.174	0.652	0.941	0.923
s_r	0.411	0.440	0.542	0.465
s_b	1.162	0.627	0.915	0.902

[a] 180 analyses.
[b] Data from Collaborator 10 omitted.

graduations. For pork samples, continue boiling solvent in flask additional 15 min before proceeding with fat determination.

Fat Determination

Use disposable pipet to return solvent layered on water in distillation receiver to extract in boiling flask. Cool flask and contents to room temperature with tap water. Transfer 20 ml aliquot of extract with class A pipet to tared tall-form beaker. Evaporate solvent in well ventilated hood by placing beaker on hot plate (ca 200°C) under stream of nitrogen directed on extract until only fat remains (6–15 min, depending on boiling point of solvent). Cool beaker (in prechilled aluminum chamber or precooled desiccator) to room temperature and weigh residue as fat. Calculate % fat = (g fat × 5 × 100)/10.

(*Note:* To remove solvent from extract by distillation, transfer aliquot to suitable, tared vessel. Distill all solvent until only fat remains; cool and weigh vessel as in evaporation procedure.)

Results and Discussion

Tables 1–3 summarize the collaborators' results from moisture determinations in 3 meat products by azeotropic distillations in cumene, *m*-xylene, and ethylbenzene. Tables 4–6 summarize the results for fat determinations in the same samples. Each table reports the means of 6 replicate analyses, the ranks of the means as a basis for collaborators' scores, and the error statistics calculated from the collaborative results with the methods. Because one collaborator (No. 10) did not report moisture data for cumene and *m*-xylene, Tables 1 and 2 list only 9 means for each meat product and the possible range of collaborators' scores is 3 to 27 instead of 4 to 29. Results from Collaborator 10, which were consistently low for moisture and consistently high for fat, suggest that a systematic error caused the differences. Data from Collaborator 10, therefore, were omitted from final statistical evaluation of the data in Tables 1 and 2 (moisture) and Tables 4–6 (fat). Summary statistics shown in all of the tables, except Table 3, are presented in 2 parts: intermediate results, calculated by using all the data, and final results, calculated by omitting outliers. Numerical values of s_d, s_r, and s_b calculated for the 3 meat products were not proportional to grand means of moisture or fat; errors, therefore, were not proportional to the levels of fat and moisture in the products.

Table 5. Collaborative results for fat analysis by azeotropic distillation in *m*-xylene for 3 meat products

	Fat,[a] %									
	Ground beef		Frankfurter		Pork sausage		Ranked results			Coll.
Coll.	$\bar{x}$	s_r	$\bar{x}$	s_r	$\bar{x}$	s_r	B	F	P	score
1(AR)	17.84	0.193	27.26	0.192	41.82	0.464	6	5	8	19
2	19.63	0.836	29.18	2.612	42.15	0.957	1	2	5	8
3	18.77	0.266	27.87	0.234	44.33	0.446	3	4	2	9
4	16.72	0.544	26.35	0.289	41.13	0.796	10	10	10	30
5	18.47	0.140	27.25	0.120	41.92	0.396	4	6	6	16
6	17.67	0.207	28.78	0.303	43.48	0.524	9	3	3	15
7	17.75	0.274	26.77	0.363	41.78	0.411	7	9	9	25
8	18.15	0.399	27.22	0.180	43.42	0.477	5	7	4	16
9	17.72	0.352	27.05	0.362	41.91	0.484	8	8	7	23
10	19.32	0.618	42.92[b]	7.839	47.05	1.809	2	1	1	4

Statistic	Beef	Franks	Pork	Average
Intermediate Results, Per Cent Fat				
Grand mean, $\bar{\bar{x}}$	18.20	29.06	42.90	–
Range	−2.14 to 2.70	−3.09 to 20.74	−3.28 to 6.50	–
s_d	0.865	4.946	1.762	2.525
s_r	0.436	2.402	0.793	1.211
s_b	0.846	4.828	1.732	2.469
Final Results,[c] Per Cent Fat				
Grand mean, $\bar{\bar{x}}$	18.08	27.53	42.44	–
Range	−2.02 to 2.82	−1.56 to 5.07	−2.82 to 2.46	–
s_d	0.818	0.927	1.049	0.932
s_r	0.411	0.907	0.580	0.633
s_b	0.801	0.850	1.022	0.891

[a] 179 analyses.
[b] Collaborator 10 reported 5 replicate values only.
[c] Data from Collaborator 10 omitted.

Table 6. Collaborative results for fat analysis by azeotropic distillation in ethylbenzene for 3 meat products

	Fat,[a] %									
	Ground beef		Frankfurter		Pork sausage		Ranked results			Coll.
Coll.	$\bar{x}$	s_r	$\bar{x}$	s_r	$\bar{x}$	s_r	B	F	P	score
1(AR)	17.42	0.365	28.45	0.193	41.62	0.411	6	2	9	17
2	18.32	0.958	28.35	0.740	42.72	0.757	3	3	5	11
3	18.70	0.429	27.53	0.489	44.08	0.133	2	5	2	9
4	17.08	0.599	26.08	0.669	41.52	1.029	9	10	10	29
5	18.17	0.140	26.95	0.229	42.29	0.230	4	8	6	18
6	17.16	0.419	27.80	0.295	43.06	0.574	8	4	4	16
7	16.66	0.302	26.74	0.189	41.63	0.604	10	9	8	27
8	17.63	0.701	27.25	0.274	43.43	0.499	5	6	3	14
9	17.38	0.461	27.20	0.154	41.70	0.484	7	7	7	21
10	20.52	2.557	29.10[b]	1.445	48.30	3.677	1	1	1	3

Statistic	Beef	Franks	Pork	Average
Intermediate Results, Per Cent Fat				
Grand mean, $\bar{\bar{x}}$	17.90	27.54	43.04	–
Range	−1.66 to 6.90	−2.03 to 3.26	−3.03 to 12.06	–
s_d	1.109	0.901	2.046	1.353
s_r	0.955	0.540	1.287	0.928
s_b	1.038	0.868	1.978	1.295
Final Results,[c] Per Cent Fat				
Grand mean, $\bar{\bar{x}}$	17.61	27.37	42.45	–
Range	−1.37 to 2.49	−1.86 to 1.73	−2.44 to 1.75	–
s_d	0.660	0.759	0.929	0.783
s_r	0.536	0.414	0.582	0.511
s_b	0.622	0.740	0.898	0.754

[a] 178 analyses.
[b] Collaborator 10 reported 4 replicate values only.
[c] Data from Collaborator 10 omitted.

Average estimates of error for both moisture (Tables 1–3) and fat (Tables 4–6) were higher than those for the AOAC methods, and, therefore, the resulting expected standard errors calculated for all solvents were also higher. For moisture, the value was about 1.5 times the value of ±0.637% calculated for the AOAC method. For fat, the value was about 2 times the value of ±0.565% obtained for the AOAC method:

	Expected Std Error	
Solvent	*Moisture, %*	*Fat, %*
Cumene	0.99	1.01
m-Xylene	0.81	1.09
Ethylbenzene	1.03	0.91
	—	—
Av.	0.94	1.00

The results of analysis with each solvent were compared, by means of linear regression, with results from the AOAC methods given in refs. 4 (moisture) and 6 (fat); *see* Table 7. *F*-Values showed that both moisture and fat results, with any of the 3 solvents, differed significantly from results by the AOAC methods at the 1% probability level. All slopes and correlation coefficients approached 1.000. For data on moisture, all intercepts were negative, which suggested the source of the high *F*-values. Apparently the azeotropic distillation method tended to underestimate moisture content by an average of 1% for all 3 products. For data on fat, the fact that all intercepts were positive while the slopes were slightly less than 1.000 indicated that the mean fat values were determined quite accurately for ground beef and frankfurter but the values for pork sausage were underestimated by about 1.5%.

Comments of Collaborators

Collaborator 3 felt that the use of a hot plate to evaporate the solvents was very time consuming. He found that, to prevent overheating the fat residues, constant attention was required

Table 7. Computations for comparison of rapid methods for analysis of moisture and fat and official methods[a]

Solvent	Values from linear regression: Intercept	Slope	Sum of squares, SS_R	Corr. coeff.	Slope=1 Intercept=0 Sum of squares, SS_E	F-value[b]
			Moisture Analysis			
Cumene	−1.536	1.016	17.23	0.995	28.0	6.91
m-Xylene	−0.547	0.997	15.00	0.996	27.2	8.95
Ethylbenzene	−0.798	0.998	31.69	0.993	56.6	11.00
			Fat Analysis			
Cumene	0.889	0.949	22.39	0.995	40.4	10.04
m-Xylene	1.329	0.942	20.52	0.996	34.6	8.60
Ethylbenzene	0.613	0.960	17.78	0.996	32.0	9.97

[a] Data from Collaborator 10 omitted.
[b] All values are significant at the 1% level.

to determine the point at which all solvent was removed. He admitted that the method provided results more rapidly than the official method but more analytical time was required and, therefore, he would not recommend this method.

Collaborator 6 mentioned that relatively high boiling temperature of the solvents required care during evaporation of the extract to prevent fat decomposition.

Collaborator 8 preferred accurately weighing about 10 g sample to weighing 10.00±0.01 g, because the former is less time consuming. He also used receivers without solvent return tubes.

Recommendation

It is recommended that the method described for determining moisture and fat in the same sample, which may be useful as a rapid screening procedure for meats prior to processing or for finished products where rapid analysis is more important than maximum accuracy and precision, be adopted as official first action.

Acknowledgments

The Associate Referee expresses his appreciation to the collaborators and their associates, all of whom were cited in the first report of this series (4), for their cooperation and participation in this study. Special thanks are also extended to Virginia G. Metzger, Brandt Kramer, and Ruth D. Zabarsky of this laboratory for their assistance in processing the collaborative data.

References

(1) Pettinati, J. D., Swift, C. E., & Cohen, E. H. (1973) *JAOAC* 56, 544–561
(2) *Official Methods of Analysis* (1970) 11th Ed., AOAC, Washington, D.C., secs. 24.003(a) and (b), 24.005(a)
(3) Cohen, E. H., & Kimmelman, C. P. (1972) *JAOAC* 55, 578–580
(4) Pettinati, J. D., Metzger, V. G., Van Horn, D., & Cohen, E. H. (1973) *JAOAC* 56, 1130–1139
(5) Cohen, E. H. (1971) *JAOAC* 54, 212–214
(6) Pettinati, J. D., Swift, C. E., & Cohen, E. H. (1973) *JAOAC* 56, 1140–1143

The recommendation of the Associate Referee was not approved by the General Referee and by Subcommittee C and was not adopted by the Association; *see* (1973) *JAOAC* 56, 399–400. The Association felt that a study of current screening methods should be conducted before additional screening methods are adopted.

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

This report of the Associate Referee, E. H. Cohen, was presented at the 86th Annual Meeting of the AOAC, Oct. 9–12, 1972, at Washington, D.C.